1: Virology. 1998 Feb 1;241(1):101-11.

Studies of the binding properties of influenza hemagglutinin receptor-site mutants.

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Site-specific mutations have been made in the influenza hemagglutinin (HA) receptor binding site to assess the contribution of individual amino acid residues to receptor recognition. Screening of mutant HAs, expressed using recombinant vaccinia virus-infected cells, for their abilities to bind human erythrocytes indicated that substitutions involving conserved residues Y98F, H183F, and L194A severely restricted binding and that the substitution W153A prevented cell surface expression of HA. Mutation of residues E190 and S228 that are in positions to form hydrogen bonds with the 9-OH of sialic acid appeared to increase erythrocyte binding slightly, as did the substitution G225R. Substitutions of other residues that are directly or indirectly involved in receptor binding, S136T, S136A, Y195F, G225D, and L226P, had intermediate effects on binding between these two extremes. Estimates of changes in receptor binding specificity based on inhibition of binding to erythrocytes by nonimmune horse sera indicated that mutants G225R and L226P, unlike wild-type HA, were not inhibited; Y195F and G225D mutants were, like wild type, inhibited; and erythrocyte binding by mutants S136A, S136T, E190A, and S228G was only partially inhibited. Viruses containing mutant HAs Y98F, S136T, G225D, and S228G that cover the range of erythrocyte binding properties observed were also constructed by transfection. All four transfectant viruses replicated in MDCK cells and embryonated hens' eggs as efficiently as wild-type X-31 virus, although the Y98F mutant virus was unable to agglutinate erythrocytes. Mutant MDCK cells that have reduced levels of cell surface sialic acids were susceptible to infection by S136T, G225D, and S228G transfectant viruses and by wild type but not by the Y98F transfectant virus.